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RESEARCH NOTE . . .

LEISHMANIASIS IN TEXAS: ISOLATION OF *Leishmania mexicana* FROM *Neotoma micropus*

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A female Neotoma micropus infected with Leishmania was collected in Zavala County, Texas, on January 15, 1990. The infection was limited to lesions at the bases of the ears, and the parasite grew readily in Schneider's Drosophila medium supplemented with 20% fetal bovine serum. Isozyme analysis determined the parasite to be L. mexicana.

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The leishmaniases, a complex of parasitic diseases, are a serious public health problem in endemic areas of Africa, Asia, the Mediterranean region, Central and South America, and southern Mexico. Reports of cutaneous leishmaniasis in humans in southern Texas and adjacent Mexico indicate that an enzootic focus also exists in this region, but the vertebrate reservoir(s) and sand fly vector(s) remain unknown (Grimaldi et al., 1989). We report the first isolation of Leishmania from a rodent host collected in Texas.

In late June, 1989, an elderly, female resident of Zavala County, Texas, was diagnosed as having cutaneous leishmaniasis. Although confirmed histologically, the parasite was not isolated in culture, and a specific identification was not made. The woman had lived at her present residence for the previous 3 years and rarely left except to attend a nearby church.

Extensive live-trapping for small mammals was conducted around the case residence in an effort to collect potential hosts of Leishmania. When trapping at the residence proved unsuccessful, the traps were relocated to a site approximately 2 km from the residence where rodent activity was apparent. Emphasis was given to collecting specimens of Neotoma micropus, the southern plains woodrat. This cricetid rodent was considered a likely zoonotic host since 1) its range (Davis, 1974) matched the distribution of human leishmaniasis cases in Texas, 2) Lutzomyia anthophora, a sand fly which has transmitted Leishmania mexicana under laboratory conditions (Endris et al., 1984), commonly was found in N. micropus nests (Young, 1972), 3) cricetid rodents are hosts for L. mexicana in other endemic areas (Lainson and Shaw, 1979), and 4) the senior author had seen evidence of woodrats within 200 m of 4 other case residences.

On January 15, 1990, 3 female N. micropus were collected. Two females (weights 180 g and 230 g) were apparently normal with no demonstrable

Leishmania infection. The pinnae of both ears on the remaining female (220 g) were completely eroded, with multiple, irregularly-bordered lesions covering the cauliflower-like swellings at the bases of the ears (Fig. 1). Numerous Leishmania amastigotes were seen in impression smears of lesion tissue stained with either Giemsa or genus-specific fluorescent-labeled monoclonal antibodies. Only 1 amastigote was seen in 200 fields of a fluorescent-stained impression smear of liver. Giemsa-stained impression smears and cultures of liver and spleen tissue were negative for Leishmania.

The primary isolation of parasites was made from a biopsy sample placed in Schneider's Drosophila medium (GIBCO Laboratories Inc., Grand Island, New York) supplemented with 20% (v/v) heat-inactivated fetal bovine serum (GIBCO), 500 units/ml penicillin, and 500 ug/ml streptomycin. The isolate (MNEO/US/90/WR972) was passed in the supplemented Schneider's medium until sufficient promastigotes were available for isoenzyme analysis.

Isozyme patterns were determined by cellulose acetate electrophoresis (Kreutzer and Christensen, 1980). The isolate was characterized twice for up to 20 enzymes including the 3 enzymes known to accurately identify parasites of the genus Leishmania: glucose phosphate isomerase (GPI-EC 5.3.1.9), mannose phosphate isomerase (MPI-EC 5.3.1.8), and phosphogluconate dehydrogenase (6PDGH-EC 1.1.1.44) (Kreutzer et al., 1987). The specific identification was based on comparison with World Health Organization reference strains which were run in parallel.

The apparent restriction of the parasites to the ears, the ease of isolation and rapidity of growth in culture, and its sensitivity to temperatures > 37 C are all suggestive of L. mexicana. Isozyme characterization determined that the parasite isolated from N. micropus collected in Texas is L. mexicana.

Additional studies to detail other biological and biochemical characteristics of the isolate are under way.

If N. micropus is a zoonotic reservoir of Leishmania in Texas, L. anthophora is a likely candidate for vector, at least among woodrats. Based on her weight, the age of the infected female was estimated to be 4-5 months (Raun, 1966). This estimate and the condition of the lesions suggest that transmission of Leishmania occurred in the area during the fall of 1989. Transmission in September through November is consistent with the seasonal activity patterns of L. anthophora (Addis, 1945). Other vertebrates such as opossums, hispid cotton rats, and armadillos and other sand flies such as Lutzomyia diabolica and Lutzomyia texana also may be involved in the enzootic cycle in Texas, but humans are undoubtedly accidental hosts. Additional studies to quantify the prevalence of Leishmania infections in Neotoma populations, document the involvement of L. anthophora, identify other reservoirs, determine the vector of Leishmania to humans, and detail the factors which lead to spillover of Leishmania into human populations are under way.

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FIGURE 1. *Leishmania*-infected *Neotoma micropus* collected in Zavala County, Texas, on January 15, 1990.